AWARD NUMBER: W81XWH-15-1-0295

TITLE: A Role of Plasminogen in Promoting the Immune Escape in Small Cell Lung Cancer

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REPORT DATE: November 2016

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE November 2016	**2. REPORT TYPE** Final	**3. DATES COVERED** 1 Sep 2015 - 31 Aug 2016

4. TITLE AND SUBTITLE

A Role of Plasminogen in Promoting the Immune Escape in Small Cell Lung Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER W81XWH-15-1-0295

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Pittsburgh
Pittsburgh, PA 15213

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This project tests the hypothesis that the plasminogen activation system is involved in immune escape and immune suppression in SCLC by developing a protective "coat" around the tumor cells, consisting of the plasmin, PAS and the fragments of the immunoglobulin G (IgG)s, that are cleaved by Plasmin in CH1-CH2 и CH2-CH3 domains of Fc fragments. The results of the study demonstrated that this protective coat is expressed on the surface of tumor cells, and that the level of its expression is significantly higher in metastatic tumor cells than in primary tumor cells and normal tissue cells.

The results of this project have generated an important new fundamental knowledge about lung tumor biology. Further studies may lead to development of novel therapeutic approaches based on the dissociation of the protective "coat" from the tumor cell surface to improve the outcome of immunotherapy.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	10	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Page No.

1. Introduction

This project was designed to test the idea that the plasminogen activated system (PAS) helps small cell lung cancer cells to avoid immune surveillance and supports cancer progression. This project has relevance to the “Understanding the molecular mechanisms of progression to clinically significant cancers” initiative. We hypothesize that the plasminogen activation system is involved in immune escape and immune suppression in SCLC by developing a protective “coat” around the tumor cells, consisting of the plasmin, PAS and the fragments of the immunoglobulin G (IgG)s, that are cleaved by Plasmin in CH1-CH2 и CH2-CH3 domains of Fc fragments.

2. Keywords

Small cell lung cancer, plasminogen, plasmin, immunoglabulins, plasminogen activating system, urokinase, urokinase-type plasminogen activator (uPA), Urokinase receptor (uPAR), metastasis, mmune surveillance

3. Accomplishment

The major long term goal of this project is to develop new adjuvant immunotherapy of SCLC capable of targeting tumor cells, establishing immune surveillance, and delaying or preventing tumor recurrence. The plasminogen and plasminogen-activated system (PAS) including Urokinase and the receptor for the urokinase plasminogen activator (uPAR) is correlated with aggressive/metastatic disease in lung cancers [1, 2]. During this pilot study we tested our hypothesis that plasminogen-activated system plays a key role in the aggressive phenotype of small cell lung cancer by helping cancer cells to avoid immune surveillance and supports cancer progression to metastasis.

To test for the ability of plasmin to cleave IgGs, we isolated plasminogen from human blood samples.

To generate plasmin, the active form of plasminogen, we incubated plasminogen with the enzyme urokinase (uPA). Glu-plasminogen was extracted from frozen blood of donors with the affine chromatography method using Lys-sepharose 4B column at 4 C and ph 8.0. Next, we used this activated plasmin to investigate its ability to cleave immunoglobulins (Figure 1). We found that approximately 30% of immunoglobulins were cleaved by plasmin. Further studies, with adjusted incubation parameters, need to be performed to determine if the majority of immunoglobulins can be cleaved. If we find that truly only 30% of immunoglobulin can be cleaved, what are the determining characteristics that control this process? Using plasminogen as the antigen we further tested the ability of naïve and cleaved immunoglobulins to bind to plasminogen. Importantly, we found that cleaved immunoglobulins bind to plasminogen at a rate approximately 5 times higher than that found for naïve, not cleaved immunoglobulins.

We found that the binding capacity of cleaved immunoglobulins was associated with the presence of the C terminal amino acid Lysine. The presence of C- terminal Lysine was evaluated using carboxipeptidase A and ELISA to test for affinity to plasminogen. We quantified plasma levels of plasmin, plasminogen and immunoglobulins and confirmed that the level of plasmin, plasminogen, and immonoglobulins is significantly higher in plasma from cancer patients than from healthy donors. Using tissue samples and immunohistochemistry we found that the expression of plasminogen activating system (uPA, uPAR etc) is significantly higher in tumor cells than in normal healthy cells and that coexpression of uPAR, plasminogen and immunoglobulins was higher in cancer cells.

Next we evaluated small cell lung cancer cell lines that were generated from tumor samples of the untreated patients with early stages of SCLC (DMS-114, DMS-53) and from the heavily treated patients with the metastatic and drug resistant SCLC (H69, H345 and H82). uPAR receptors are critical component of the plasminogen activated system, binding of plasminogen to uPAR is necessary for the activation of plasminogen to plasmin. The uPA receptor is anchored to the plasma membrane, localizing the uPA system to the cell surface [3]. High expression of uPAR on the invasive front of tumors facilitates invasion and other roles in cellular migration [4]. Using flow cytometry methods we found that the expression of the uPAR receptors was significantly higher in H69, H345, H82 metastatic SCLC cells, than in DMS-114 and DMS-53 cells (Figure 2). Using fluorescent activating cell sorting we isolated H69 cells with low and high expression of uPAR on the cell surfaces. High expression of uPAR receptors was associated with co-expression of DNA-PK (Figure 3). Next we cultured uPAR+ and UPAR – cell in culture media supplemented with human IgGs (0.1mg/ml) for 1 hour and then determined residual concentration of IgGs using column chromatography. We determined that uPAR-positive cells accumulated a protective “coat” around them, whereas cells with no or low expression of uPAR have a small amount of attached IgGs.

Summarizing, we have determined that tumor cells have a protective “coat” around them (consisting of plasmin and the fragments of the immunoglobulin G (IgG)s, that are cleaved by Plasmin) and that the more advanced SCLC cells have a higher expression of uPAR and of this protective “coat”.

4. Changes/Problems

Because of the limited availability of SCLC tumor samples, we were not able to determine the impact of the protective “coat” on SCLC immune surveillance. Therefore, we used slides of frozen prostate tumor samples and immunohistochemical staining of IgGs to detect the level of association with tumor tissues. As presented in Figure 4, tumor tissues were shown to bind significantly higher amounts of immunoglobulin than normal tissues. These levels were dramatically higher than was found bound to adjacent normal tissue cells.

5. Impact

The result of this project was the generation of an important new fundamental understanding about lung tumor biology. Further studies may lead to development of novel therapeutic approaches based on the dissociation of the protective “coat” from the tumor cell surface before DC based vaccine preparation, or depletion of cleaved IgGs from patients’ plasma using extracorporeal hemacorrection (plasmapheresis) before immune therapy.

6. Products

Results of the studies will be published. Manuscript is under preparation.

7. Participants

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Consultant: Mark Socinsky, MD; a Director of Specialized Program of Research Excellence (SPORE) in Lung Cancer the University of Pittsburgh Cancer Institute (UPCI).

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8. Special reporting requirements

None

9. References

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10. Attachments

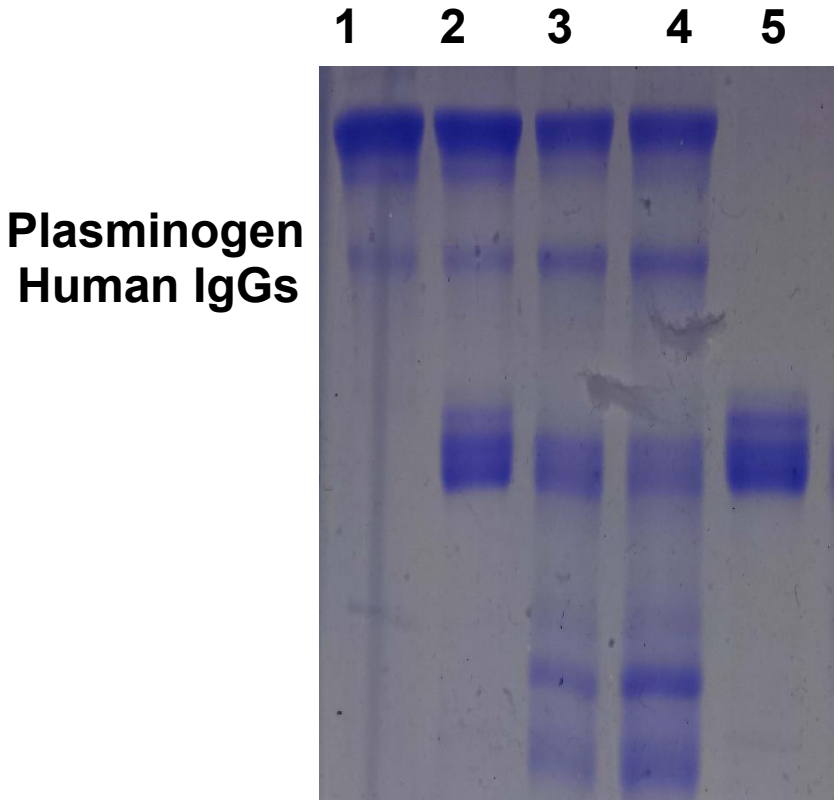

Figure 1. Western blot analysis of cleaved IgGs generated by activated plasmin.

Plasminogen is the inactive precursor of trypsin-like serine plasmin. When it becomes activated, it is converted to plasmin. The generation and activation of plasmin requires the binding of urakinase (uPA) to plasminogen.

1. Plasminogen. 2. Plasminogen + human IgGs. 3. Plasminogen + uPA were incubated for 20 min and then IgGs were added to probe. 4. Plasminogen + uPA were incubated for 120 min and then IgGs were added to probe. 5. Human IgGs

Human IgGs (1mg/ml), Plasminogen (500mg/ml), uPA (300 ed/ml). 20 ml probes of proteins were used.

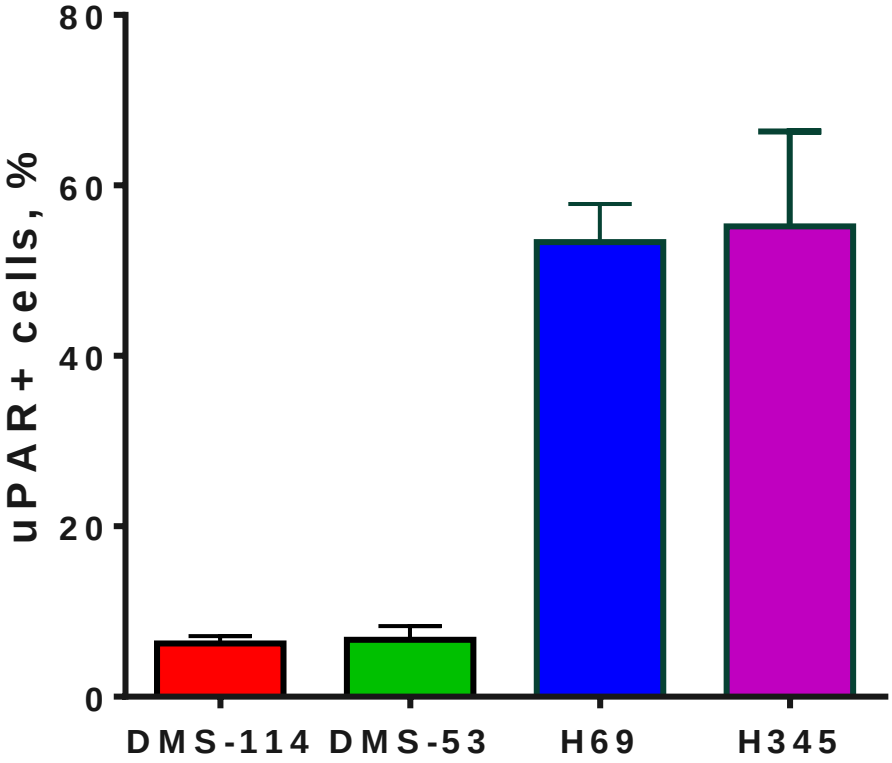

Figure 2. Flow cytometry analysis of the expression of uPAR receptors in SCLC cells.

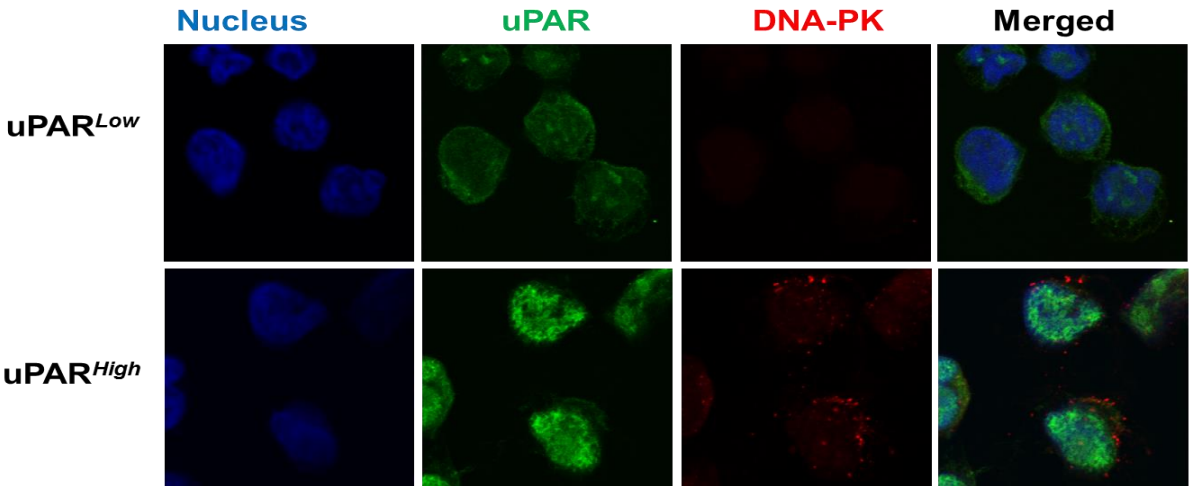

Figure 3. Confocal microscopy images of H69 SCLC cells with low and high level of uPAR receptors expression. Representative images of uPAR^{low} and uPAR^{high} cells stained with DAPI (nucleus, blue), with Alexa488 conjugated anti uPAR antibody (green) and anti DNA-PK antibody conjugated with Alexa687 (red).

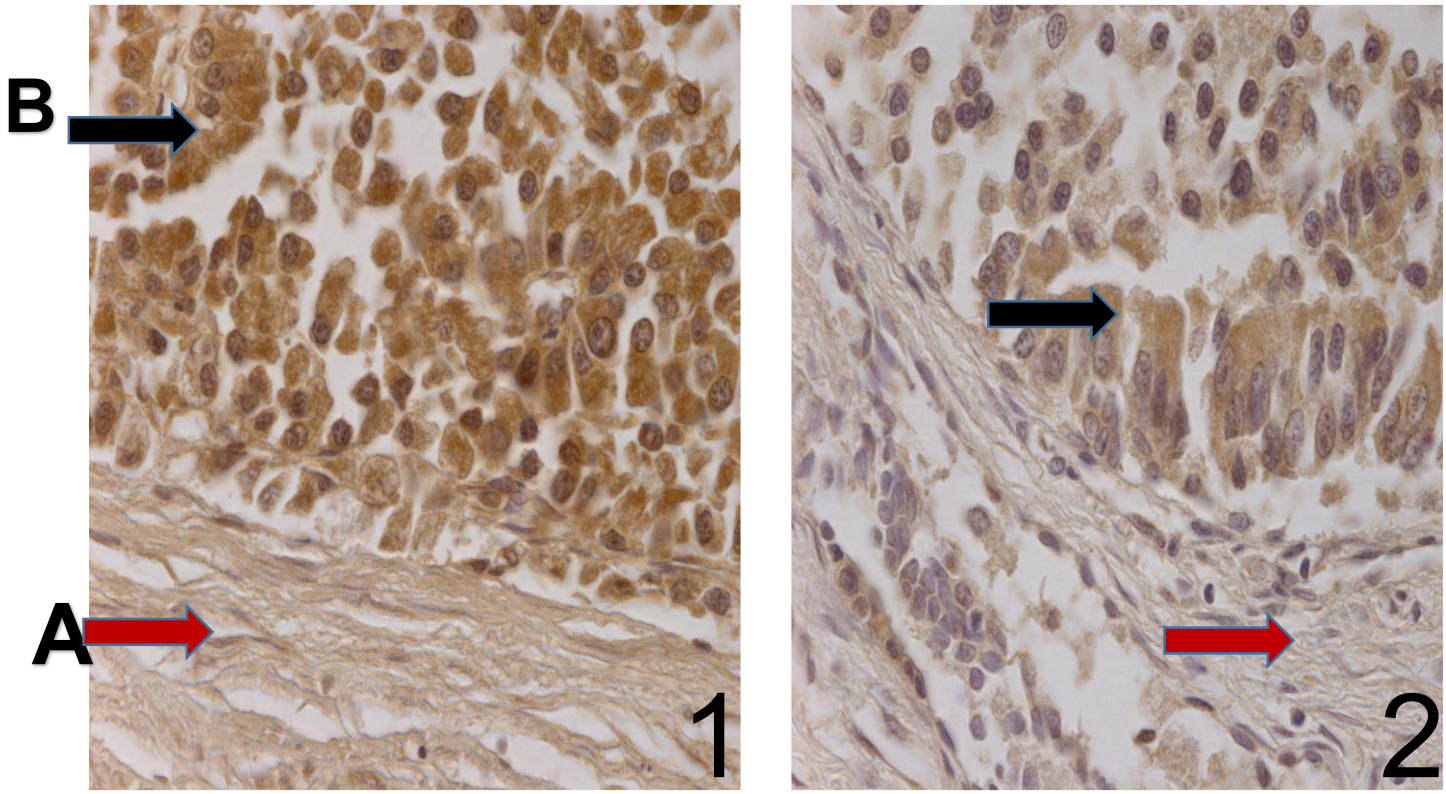

Figure 4. Immunohistochemical staining of immunoglobulins in human prostate cancer samples from 2 patients. Representative images of peroxidase labelled IgGs are shown. Low expression of IgGs in healthy tissue cells (A), while tumor tissue cells “accumulate” immunoglobulins (B).